AMENDMENS

Claims 1-6 and 8-25 are pending.

Claims 4, 12, 14 and 15 have been amended.

Claims 1-3, 5, 8-9, 11, and 13 have been withdrawn.

Claim 7 has been canceled.

Support for the amendments is found in the claims and specification (e.g., page 10, ln. 9-17 and Table 4), as originally filed.

No new matter is believed to have been added.

REMARKS/ARGUMENTS

Aging of cells of the skin causes changes in appearance, including formation of wrinkles, sagging of the skin, and loss of the skin elasticity (page 1 of the specification). The claimed method is based on the finding of the relation between the force generated in skin fibroblasts, i.e. non-muscle cells, and aging. The inventors have found that the force generated by skin fibroblasts (i.e., non-muscle cells) is reduced with aging, and that the expression of an enzyme which phosphorylates myosin light-chain, i.e. Rho kinase or myosin light-chain kinase, is reduced in aged skin fibroblasts. The present inventors have also found that a substance capable of enhancing the expression level of such an enzyme can prevent aging of the skin such as sagging of the skin, loss of skin elasticity, or wrinkle formation, or improve the skin.

Claims 4, 6, 10, 12, and 14-17 are rejected under 35 U.S.C. 103(a) over Lopes, US 5,942,478, and the Sigma Aldrich Catalogue (1996). The rejection is traversed because

(1) "agar-agar" is different from *Digene simplex;*

(2) the claimed method provides an advantageous enhancement of the expression level of Rho kinase or myosin light-chain kinase compared to that of Lopez and Sigma;

(3) the combination of the references does not describe or suggest (i) a treating agent capable of increasing the force generated by skin non-muscular cells, wherein the skin non-muscular cells are skin fibroblasts (as in claims 19-20 and 22-23); and (ii) an amount of the extract as reduced to dry weight is 0.00001% wt. to 0.01% (or to 0.002 %) based on the total amount of said agent (as in claims 24-25).

(4) In addition, one would not have been motivated to select a subject in need of a treatment of wrinkles, sagging skin, and/or a loss of the skin elasticity, and to apply the Lopes soap with a reasonable expectation of increasing the force generated by skin non-muscular cells (increasing the level of Rho kinase and/or myosin light-chain kinase) and treating or improving wrinkles, sagging skin, and/or a loss of the skin elasticity.

"Agar-agar" is different from *Digene simplex*

As described on page 1 of Sigma, Agar is a polysaccharide complex obtained through bleaching and hot water extraction of agarocytes from the red alga *Rhodophyceae*, found in the Pacific and Indian Oceans and in the Sea of Japan. The genera *Gelidium, Acanthopeltis, Ceramium, Pterocladia* and *Gracilaria* predominate in the agar production. Agar is composed of about 70% agarose and 30% agaropectin, and agarose is composed of galactose and anhydro-galactose (Fig 1 of Sigma).

Therefore, agar (or agar-agar) is generally prepared from hot water extracts of *Gelidium*, *Acanthopeltis*, *Ceramium*, *Pterocladia* or *Gracilaria*, and is mainly composed of galactose.

The active ingredient used in the claimed method is an aqueous ethanol extract of *Digenea simplex*. *Digenea simplex* is seaweed belonging to order *Ceramiales*, genus *Rhodomelaceae*. The toxonomy pages submitted previously show that agar is generally produced from small red algae belonging to the order *Gelidiales* which is different form the order *Ceramiales* to which *Digenea simplex* belongs.

The aqueous ethanol extract of *Digenea simplex* do not contain galactose as polysaccharides such as galactose is not extracted with aqueous ethanol.

Therefore, an aqueous ethanol extract of *Digenea simplex* used in the claimed method is different from agar-agar used in Lopes or agar available from Sigma.

Further, the enhancing effect of the aqueous ethanol extract of *Digenea simplex* on the expression level of Rho kinase or MLC kinase is advantageous compared to the effect of the Lopes composition since agar-agar does not enhance the expression level of Rho kinase as shown below.

Applicants have investigated the effect of agar-agar on the expression level of Rho kinase in fibroblasts. Agar-agar (Sigma, Cat. no. A1296-100) was dissolved in warm DMEM to prepare a 0.1% (evaporation solid residue %) solution. The solution was sterilized by filtration with a sterilizing filter and then diluted to 0.01% and 0.002% (evaporation solid residue %). Preparation of fibroblast cultures, administration of the agar-agar solutions to the fibroblasts and detection of the expression level of Rho kinase were conducted as described in the Examples of the present specification. The control sample is as in Table 2 of the present specification. As shown in the table below, the expression level of Rho kinase was not enhanced by agar-agar.

	Concentration (evaporation solid residue %)	Rho kinase relative expression (%)
CONTROL		100
agar-agar	0.002%	89.36
agar-agar	0.01%	91.31

The claimed method provides advantageously increased levels of expression of Rho kinase or MLC kinase compared to a control (the protein expressed in the youngest cells (Passage number 7) taken as 100%). See page 19 of the present specification. *See also* the Declaration of Tsutomu Fujimura submitted herewith.

Further, Lopes does not describe a treating agent capable of increasing the force generated by skin non-muscular cells, wherein the skin non-muscular cells are skin fibroblasts (as in claims 19-20 and 22-23).

Lopes does not describe selecting a subject in need of a treatment and applying the claimed agent for treating aging skin.

Even if the lubricant and skin-protecting ingredients of the Lopes soap can lubricate the skin and, possibly, improve the skin, the effect is expected to be cosmetic. Lopes does not describe that the lubricant and a skin-protecting ingredients of a soap can treat (and are intended to treat) the aging skin which has wrinkles, is sagging, and/or lost elasticity.

There is insufficient nexus between a cosmetic effect (lubricating the skin) and a therapeutic effect (treating the aging skin). The need for lubricating may be caused by ingredients used in a soap to improve feel after using the soap (e.g., because of other soap components cause dry feeling), while the claimed method is directed to treating a subject in need of improving wrinkles, is sagging, and/or lost elasticity.

A soap of Lopez is a washable composition providing microbicidal and sanitizing effects, while in the claimed method, the skin treating agents are applied to the skin for treatment (i.e., not washable).

Lopes describes a soap composition comprising 0.1-25 wt. %. of agar-agar (col. 2, lines 13-26). The references do not describe an amount of the extract as reduced to dry weight is 0.00001% wt. to 0.01% (or to 0.002 %) based on the total amount of said agent (as in claims 24-25).

One would not have been motivated to select a subject in need of a treatment of wrinkles, sagging skin, and/or a loss of the skin elasticity, and to apply the Lopes soap with a reasonable expectation of increasing the force generated by skin non-muscular cells and treating or improving wrinkles, sagging skin, and/or a loss of the skin elasticity.

Lopes describes a soap composition comprising agar-agar, wherein agar-agar is added as a skin-lubricating and skin-protecting agent (abstract, col. 4, line 64 to col. 5, line8; col.2, lines 13-26).

One would not have been motivated to select a subject in need of a treatment of wrinkles, sagging skin, and/or a loss of the skin elasticity, and to apply the Lopes soap with a reasonable expectation of (a) treating or improving wrinkles, sagging skin, and/or a loss of the skin elasticity, and/or (b) increasing the force generated by skin non-muscular cells because (i) there is insufficient nexus between a cosmetic effect (lubricating the skin) and a therapeutic effect (treating the aging skin), (ii) Lopez's washable composition provides microbicidal and sanitizing effects, while in the claimed method, the skin treating agents are applied to the skin for treatment, and (iii) Lopes does not describe selecting a subject in need of a treatment and applying the claimed agent for treating aging skin.

Thus, Lopez and the Sigma Aldrich Catalogue do not make the claimed method obvious.

Applicants request that the rejection be withdrawn.

Claims 24 and 25 are rejected under 35 U.S.C. 112, first paragraph, for lack of written description. Applicants respectfully traverse.

The present claims are directed to a method for treating aging of the skin comprising contacting a skin treating agent with the skin, wherein the agent comprises a substance that enhances the expression level of Rho kinase or myosin light-chain kinase (MLC kinase).

The present specification describes when an extract (e.g., of the plant) is incorporated as an active substance, the amount of the extract as reduced to a solid content is 0.00001 to 5 wt.%, more preferably 0.0001 to 2 wt.% (page 11, third full paragraph).

The Examples demonstrate that the claimed extracts enhance the expression level of MLC kinase and Rho kinase in a well-recognized model of the *in vitro* aged human skin fibroblasts. See pages 20-22. The Examples also confirm that the force generated by the *in vitro* aged human skin fibroblasts (old fibroblasts), through stimulation by thrombin or LPA was significantly reduced compared to the force generated by the young fibroblasts. See Example 1, pages 15-17, and figs. 1-2. Example 2 further shows that aged human skin fibroblasts express myosin phosphoenzyme, Rho kinase, short MLCK and long MLCK. See pages 18-20, Table 1. Further, Example 2 showns that in the *in vitro* aged human skin fibroblasts, expression of MLCK and Rho kinase is reduced compared with that of the young fibroblasts.

Thus, the *in vitro* aged human skin fibroblasts is a well-known and adequate model for showing that the force generated by individual fibroblasts is reduced with aging of the skin cells and that the expression level of MLC kinase and Rho kinase is reduced in the old fibroblasts and can be increased by the claimed active agents. See page 17, ln. 1-3.

The *in vitro* aged human skin fibroblasts does provide a probative value with regard to an initial dosage level so that one skilled in the art could determine a more precise dosage for the treatment without undue experimentation. *Cross et al. v. Iizuka et al.*, 224 USPQ 739, 744 (Fed. Cir. 1985). Moreover, the present specification provides a therapeutic dose and the dose used in the *in vitro* aged human skin fibroblasts is within the therapeutic dose range.

In addition, it is well known that prior to clinical studies, an activity and an initial dosage of an active compound is determined in a model (e.g., cultured cells, *in vitro* assays, animal models, etc.). *In re Brana*, 34 USPQ2d 1436, 1442-1143 (Fed. Cir. 1995); see also *Cross et al. v. Iizuka et al.*, 224 USPQ 739 (Fed. Cir. 1985); and In re Gardner, Roe, and Willey, 166 USPQ 138 (C.C.P.A. 1970). If human testing were required [instead of adequate model studies], the associated cost would prevent many companies from obtaining patent

protection on promising new inventions, thereby eliminating an incentive to pursue potential cures. *In re Brana,* at 1443.

It is well established that cell and animal models give valuable information regarding, e.g., activities and an initial dose. *Id.* If reasonably correlated to the particular therapeutic or pharmaceutical utility, data generated in *in vitro* assays or from testing in an animal model is sufficient to establish therapeutic utility for a compound, composition or process. *See* § MPEP 2107.03.

Thus, the dosage of claims 24 and 25 is adequately supported in the originally filed specification. Applicants request that the rejection be withdrawn.

This application presents allowable subject matter, and the Examiner is kindly requested to pass it to issue.

Should the Examiner have any questions regarding the claims or otherwise wish to discuss this case, he/she is kindly invited to contact Applicants' below-signed representative, who would be happy to provide any assistance deemed necessary in speeding this application to allowance.

Respectfully submitted,

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